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Light and Small Angle X-Ray Scattering and Biological Macromolecules

Light scattering is a phenomenon which we encounter constantly in our everyday experience. It is, therefore, no wonder that it has attracted the attention of thinkers over the course of several centuries. In fact, it was Leonardo da Vinci who first attributed the blue color of the sky to the interaction of light with particles present in the atmosphere. Light scattering is the cause not only of this blue color, but also of the azure hues of a southern sea and the mysterious illumination inside a glacier cave; it is to the scattering of light that we owe the glowing tones that we admire in many a sunset. Probably the earliest relation of light scattering to a biological material is due to Helmholtz who attributed the magnetism of a pair of blue eyes to nothing other than a secondary reflection of the same phenomenon.

While the discussion of phenomena due to light scattering has a history of several centuries, it is close to 100 years ago that the rigorous examination of electromagnetic scattering started, when Tyndall (*1*) in 1869 reported on experiments in which he related the intensity of scattered light to particles suspended in the atmosphere. Shortly after, in 1871, Lord Rayleigh (*2*) published his first paper on what was to establish the classical theory of light scattering. The century that followed saw an ever-increasingly detailed development of the theory and measuring techniques, with the result that today light scattering and small angle X-ray scattering have become powerful tools for the determination of molecular weights and structures of macromolecules as well as for studies of the thermodynamic and geometric aspects of their interactions. In this paper, a brief presentation of the development of the basic phenomena of light scattering and X-ray scattering, as applied to large molecules in solution, will be followed by an analysis of how these various developments have participated in the growth of the rapidly advancing field of biological macromolecules. It should be pointed out that, while historically many of the developments in light scattering and small angle X-ray scattering have been independent of each other, basically the two phenomena are identical and what applies to one will, in most cases, apply to the other. The basic difference between the two is the wave length of the radiation used (*ca.* 1.5 Å in X-ray scattering, *ca.* 4000 Å in light scattering). As a result, the molecular dimensions examined by the two techniques are of different orders of magnitude.

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Basic Phenomenon

When a beam of electromagnetic radiation strikes an electron, some of the energy is absorbed and then reemitted with the same frequency by the electron. However, whereas the incident radiation was propagated in one direction, the reemitted radiation is propagated equally in all directions, as shown in Figure 1. This is known as the scattered radiation. The

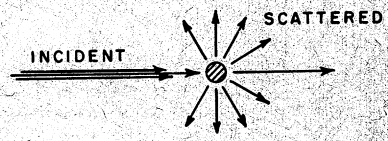

Figure 1. Schematic representation of the basic phenomenon of scattering of radiation by an electron.

intensity of scattering due to an electron, I_e, is related to the intensity of the incident beam, I_0, by the well-known Thomson equation:

$$I_e = I_0 \frac{e^4}{2r^2m^2c^4}(1 + \cos^2\theta) \qquad (1)$$

where e is the electronic charge, r is the distance between the observer and the scattering medium, m is the mass of the electron, c is the velocity of light and θ is the angle formed by the scattered and incident radiations. In the case of a particle or a molecule, the scattering is the sum of the scatterings from the constituent electrons. For a particle small with respect to the wavelength of the radiation, the scattered intensity, I_s, is given, for n particles, by the Rayleigh equation (*2*), which is closely related to equation (1):

$$I_s = I_0 \frac{8\pi^4 n\alpha^2}{\lambda' r^2}(1 + \cos^2\theta) \qquad (2)$$

where α is the polarizability and λ' is the wavelength of the radiation in the medium.

When the dimensions of the particle are not small relative to the wavelength of the radiation (a situation true for large molecules such as DNA, collagen or myosin in light scattering, and always true in small angle X-ray scattering), interference occurs between the radiation scattered from individual elements within a particle with the result that the scattering envelope (i.e., the angular dependence of the scattered radiation) is asymmetric. The reason for this is shown in Figure 2. Here we have a particle large with respect to the wavelength of the radiation. Consider scattering from elements A and B observed at points P and Q. We find that when radiation scattered by elements A and B reaches point P (in the forward direction) there is no great difference between

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the pathlengths of the two rays, so that they are not greatly out of phase with each other and interference is small. However, when the radiation scattered from A and B reaches point Q (in the backward direction) the total distance traveled by the ray from B is much greater than that from A (greater by AB + BQ − AQ), with the result that the two rays can become completely out of phase, leading to serious interference. In the forward direction, i.e., along the incident beam, scattered radiation from A and B is fully in phase, there is no interference and the observed scattering is the sum of the scatterings from all elements within the particle.

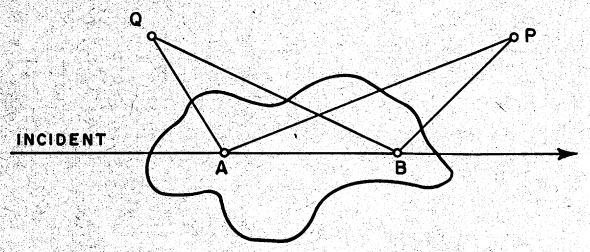

Figure 2. Scattering of radiation from a body which is not small compared to the wavelength. A and B are individual scattering elements; P and Q are points of observation in the forward and backward direction, respectively.

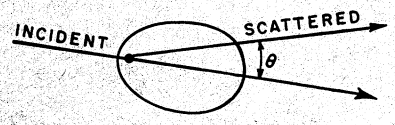

Figure 3. "Scattering envelope," i.e., angular distribution of scattering intensity from a particle sufficiently large to produce internal interference.

As a result, the scattering envelope has a shape such as shown in Figure 3. This is the reason for extrapolating the intensity data to zero angle.

For asymmetric particles, it can be shown that the angular dependence of the scattering, $P(\theta)$, varies with the orientation of the particle relative to the incident radiation. In suspension or solution, however, the particles are in constant motion, so that they assume all possible orientations as a time average. In 1915 Debye (*3*) showed that the angular dependence of the scattering from a particle of any shape, averaged over all orientations, is given by

$$P(\theta) = \frac{1}{N^2} \sum_{A}^{N} \sum_{B}^{N} \frac{\sin 2\pi s r_{AB}}{2\pi s r_{AB}} \qquad (3)$$

$$s = 2/\lambda \sin \theta/2$$

where N is the total number of scattering elements in the particle, r_{AB} is the distance between elements A and B, λ is the wavelength of the radiation and θ is the angle formed between the incident and scattered beams. From this equation, the angular dependence of the scattering of variously shaped bodies has been calculated and can be found in the literature (*4–6*). Comparison, then, of the experimental scattering envelope with calculated ones makes it possible to determine the shape of a scattering body. It is not necessary, however, to know the shape of a particle to obtain information about it. In 1939 Guinier (*7*) showed that scattering yields a characteristic geometric function of any particle which is independent of its shape, namely the radius of gyration,[2] R_G. Expanding equation (3), Guinier found that, for small values of the product $R_G s$,

$$i(s) = i(0) \exp(-4/3\pi^2(R_G)^2 s^2) \qquad (4)$$

where $i(s)$ is the scattered intensity at angle θ corresponding to a given value of s, and $i(0)$ is the intensity extrapolated to zero angle. Thus, at very low angles, a plot of log $i(s)$ versus s^2 gives a straight line, the slope of which is $^4/_3\pi^2(R_G)^2$. The intercept, $i(0)$, is found to be proportional to the square of the molecular weight.

While the Rayleigh equation (equation (2)) was derived for dilute gases, its use can be extended easily to pure liquids and solutions. In a dilute gas all particles can be regarded as entirely independent of each other and in random orientation and location. In the condensed phase, a certain amount of ordering exists; this ordering results in strong interference between the scattering from individual molecules. In a liquid, however, the molecules are in constant motion, so that the local density within a small volume element, δV, fluctuates constantly. In the case of a solution, similar fluctuations exist in the local concentration of the solute. These fluctuations are responsible for the scattering by pure liquids and solutions. Smoluchowski (*8*), Einstein (*9*), and Gans (*10*) examined this problem more than 50 years ago, but it was not until 1944 that a detailed theory of solution scattering, taking virial concentration effects into account, became available when Debye (*11*, *12*) showed that light scattering in solution was closely related to osmotic pressure.

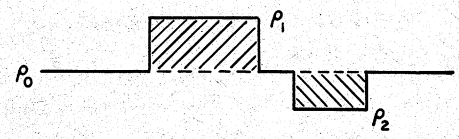

Figure 4. Schematic representation of an instantaneous electron density distribution of a scattering medium. ρ_0 is the average density; ρ_1 and ρ_2 are the densities of two volume elements, higher and lower than ρ_0, as a result of fluctuations.

In this case, both solvent and solute molecules scatter the radiation. The scattering is done by electrons and is proportional to their number; then, if the solute has a different electron density (number of electrons per unit volume) from the solvent, its introduction increases the amount of the scattering. The resultant excess scattering (Δi_s = i_s (solution) − i_s (solvent)) is given for X-rays by:

$$\Delta i_s = K'' \left(\frac{\partial \rho}{\partial C_2}\right)^2_{T,p} \delta V \overline{\Delta C_2^2} P(\theta) \qquad (5)$$

where K'' is a constant involving normalization factors, ρ is the electron density of the solution, C_2 is the solute concentration and $\overline{\Delta C_2^2}$ is the time average of the square of the concentration fluctuations. In the case of light scattering, $(\partial\rho/\partial C_2)_{T,p}$ in equation (5) is replaced by $(\partial n/\partial C_2)_{T,p}$ where n is the refractive index. Since the refractive index is a function of the electron density, the two equations reflect the same phenomenon. The density fluctuation effect is depicted in Figure 4. Here ρ_0 is the average electron density of the liquid, ρ_1 and ρ_2 those of two volume

[2] The radius of gyration is defined as the average of the distances of all elements of a body to its center of gravity.

elements, one with momentary density higher, the other lower than the average. Examination of equation (5) shows that both elements result in excess scattering. Such a fluctuation of ρ may be due also to solute concentration fluctuations. Therefore, a measurement of the refractive index increment in the case of light scattering, and a calculation (from chemical composition) of the electron density increment in X-ray scattering, together with a measurement of Δi_s give the value of $\delta V \overline{\Delta C_2^2}$. But, since

$$\delta V \overline{\Delta C_2^2} = -\frac{kTC_2\overline{V_0}}{\left(\frac{\partial \mu_0}{\partial C_2}\right)_{T,p}} \tag{6}$$

it follows that

$$\underbrace{K'\left(\frac{\partial n}{\partial C_2}\right)^2 \frac{C_2}{\Delta i(0)}}_{\text{light scattering}} = \underbrace{K''\left(\frac{\partial \rho}{\partial C_2}\right)^2 \frac{C_2}{\Delta i(0)}}_{\text{small angle x-ray scattering}} = \frac{1}{M_2 P(\theta)}\left[1 + \frac{C_2}{RT}\frac{\partial \mu_2^e}{\partial C_2}\right] \tag{7}$$

where k is Boltzmann's constant, T is the thermodynamic temperature, $\overline{V_0}$ is the partial specific volume of the solvent, μ_0 is its chemical potential, M_2 is the molecular weight of the solute, K' and K'' are optical and normalization constants for light scattering and small angle X-ray scattering, respectively, and $\mu_2^e = RT \log \gamma_2$ is the excess chemical potential of the solute, defined by

$$\mu_2 = RT \log C_2 + \mu_2^e + \mu_2^\circ (T,p) \tag{8}$$

We see that both scattering techniques can give us the molecular weight of the solute, the deviation from ideality of its solution (through μ_2^e) and its geometry, through $P(\theta)$. A positive value of the coefficient of C_2 means a predominance of repulsive forces between the polymer molecules; a negative value, to the contrary, means a net polymer-polymer attraction.

Equation (7) is perfectly rigorous for a two-component system (such as protein, component 2, dissolved in water, component 0). However, when more components are present, such as is usually the case in aqueous solutions of biopolymers which are dissolved in a medium containing supporting electrolyte (component 1), the extrapolation to zero concentration is no longer a simple function of the macromolecular molecular weight, but contains also a contribution from the interaction of components 1 and 2. This was first pointed out by Debye and co-workers (*13*) in 1946, and a rigorous thermodynamic treatment was developed shortly thereafter by Brinkman and Hermans (*14*), Kirkwood and Goldberg (*15*), and Stockmayer (*16*). For a three component system, the proper equation is:

$$K'\left(\frac{\partial n}{\partial C_2}\right)^2 \frac{C_2}{\Delta i_s} = K''\left(\frac{\partial \rho}{\partial C_2}\right)^2 \frac{C_2}{\Delta i_s} =$$

$$\frac{1}{(1+D)M_2 P(\theta)}\left\{1 + \left[\frac{\partial \log \gamma_2}{\partial C_2} - \frac{M_2}{M_1}\frac{(\partial \log \gamma_1/\partial C_2)^2}{(\partial \log \gamma_1/\partial C_1)}\right]\frac{C_2}{RT}\right\}$$

$$D = -2\alpha \frac{(\partial \log \gamma_1/\partial C_2)}{(\partial \log \gamma_1/\partial C_1)} + \left[\alpha \frac{(\partial \log \gamma_1/\partial C_2)}{(\partial \log \gamma_1/\partial C_1)}\right]^2 \tag{9}$$

$$\alpha = \frac{(\partial n/\partial C_1)_{C_2}}{(\partial n/\partial C_2)_{C_1}} \text{ (light scattering)}$$

$$\alpha = \frac{(\partial \rho/\partial C_1)_{C_2}}{(\partial \rho/\partial C_2)_{C_1}} \text{ (small angle X-ray scattering)}$$

A positive value of D means that the polymer molecules attract component 1 more strongly than solvent; conversely, a negative value of D means preferential attraction of solvent.

The equations presented above form the theoretical basis of the methods of light scattering and small angle X-ray scattering for studying the geometry and thermodynamics of macromolecules in solution. All later developments are either elaborations of these theories or their extensions to particular cases. Several important developments should be pointed out, however. These are: (1) the development by Fixman (*17*) in 1955 of a molecular theory of light scattering; (2) the introduction by Zimm (*18*) in 1948 of his well-known graphical method, in which the scattering function is plotted simultaneously as a function of concentration and $\sin^2\theta$, with extrapolation to both zero angle and zero concentration. This plot yields not only the radius of gyration and molecular weight of the molecule, but also the second virial coefficient of its solution; (3) the recent simultaneous development of small angle X-ray scattering techniques on an absolute intensity basis and their application to biological macromolecules by Kratky (*19*) and Luzzati *et al.* (*20, 21*). Such measurements have made possible the direct determination of molecular weight, as well as particle density, volume, hydration, and surface to volume ratio.

Before going on to an analysis of the impact of scattering techniques on the field of biological macromolecules, it should be emphasized that, basically, the techniques of light scattering and small angle X-ray scattering are complementary, since they represent a continuous manifestation of the same phenomenon. For thermodynamic interactions, data for use in equations (7) and (9) can be obtained by either technique, the experimental limitations making it preferable to use light scattering in the low concentration range (down to 10 mg/l), and small angle X-ray scattering above 10 g/l. In the case of geometric studies the two can be used together to great advantage, since the molecular dimensions seen by them are of different orders of magnitude; above 1000 Å for light scattering, 10–1000 Å for small angle X-ray scattering. These differences follow directly from the wave lengths of the radiations employed, and the resulting values of the parameters in equations (3) and (4).

Historical Survey of Application to Biopolymers

Measurements of protein molecular weights by light scattering go back about 30 years. Holwerda (*22*) in 1931 tried to determine the size of casein sols by this technique. In 1935, Putzeys and Brosteaux (*23*) measured carefully the molecular weights of several proteins obtaining values in good agreement with those measured by other techniques. It was the development of the detailed theory by Debye in 1944 (*11*) which gave the strong impetus to this technique, while the commercial availability of the instrument developed by Brice (*24*) made possible its widespread use in routine fashion. As a result, starting from the midpoint of this century, papers dealing with the light scattering of biological macromolecules started appearing in ever increasing volume. Among the earliest were those of Bier and Nord (*25*), in 1949, on aggregations of oval-

bumin; of Edsall and co-workers (*26*) in 1950, describing their classical studies on the applicability of the multicomponent theory to serum albumin solutions in the presence of various ions; of Halwer, Nutting, and Brice (*27*), in 1951, reporting measurements of the molecular weights of a number of proteins; and of Doty and Steiner (*28*), in 1952, reporting studies of ordering effects in serum albumin solutions under conditions of high charge and low ionic strength.

Most biopolymers are polyelectrolytes in nature and, as such, can be studied either in the presence or absence of supporting electrolytes. In either case a number of complicating factors have been demonstrated to exist and should be mentioned here. These, in turn, have been utilized to obtain important information on these molecules.

In the two-component system, if the protein is present under conditions of pH at which it carries a nonzero net charge, $\overline{Z_2}$, the Coulombic repulsion between the molecules results in ordering effects in solution. This leads to interference in the scattering, strongest at low angles. This phenomenon was first treated mathematically by Zernicke and Prins (*29*) in 1927 and by Debye and Menke (*30*) in 1930. The resulting angular dependence of the scattering is described by the equation

$$i(s) = i(0)\left\{1 - \frac{4\pi}{v_1}\int_0^\infty \left[1 - g(R)\right]\frac{\sin 2\pi sR}{2\pi sR} R^2\, dR\right\} \quad (10)$$

where v_1 is the solution volume per particle, s has its previous meaning, R is the radial distance from the center of a particle, and $g(R)$ is the radial distribution function which defines the system. Fournet (*31*, *32*; *4*), in 1949–51, introduced the Born and Green (*33*) theory of fluids into equation (10) obtaining explicit expression for intermolecular interactions. In small angle X-ray scattering experiments on concentrated protein solutions (*34*), he was able to demonstrate the presence of strong interference due to ordering in the solution. Typical small angle X-ray scattering curves for dilute and concentrated solutions of β-lactoglobulin (*35*) are presented in Figure 5, showing the presence of an interference maximum in the second case and its absence in the first. Doty and Steiner (*28*) applied the Fournet (*32*) theory to their light scattering measurements on bovine serum albumin, and tested various potentials of intermolecular interaction. Similar effects have been observed in other systems, for example, in the light scattering of ovalbumin (*36*), α-lactalbumin (*37*) and the small angle X-ray scattering of DNA (*38*).

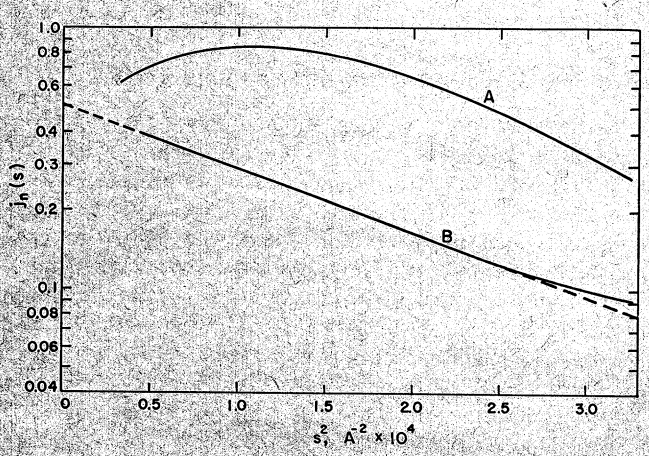

Figure 5. Guinier type plots (see equation (4)) of protein solutions which (A) exhibit intermolecular interference and (B) behave normally. A: β-lactoglobulin B solution, 1.20 g/l, pH adjusted to 2.73 with HCl, no other supporting electrolyte; B: β-lactoglobulin A, 22.8 g/l, pH 5.70, 0.1 ionic strength acetate buffer

At the isoelectric point, the average charge of a protein, $\overline{Z_2}$, is zero, but its root mean square charge, $(\overline{Z_2^2})^{1/2}$, is not. Kirkwood and Shumaker (*39*) have shown in 1952 that this causes a net attraction between the molecules, with the result that the coefficient of the concentration in equation (7) becomes itself a function of the concentration. In this case, the scattering becomes a function of the square root of protein concentration:

$$K'\frac{C_2}{\Delta i_s} = \frac{1}{M_2}\left(1 - a_1 C_2^{1/2} + a_2 C_2 + \ldots\right) \quad (11)$$

where the limiting slope, a_1, is proportional to $(\overline{Z_2^2})^{1/2}/M_2^{1/2}$. Thus, light scattering can give a measure of $\overline{Z_2^2}$. This quantity is related to the acid-base titration curve of the protein (*40*) and to its dielectric increment in solution (*41*). In a series of papers, Timasheff, Dintzis, Kirkwood, Coleman, and Tinoco (*42*, *43*) (1955–60) have verified equation (9), measured $\overline{Z_2^2}$ and further developed the theory of the second virial coefficient of an isoionic protein (*44*).

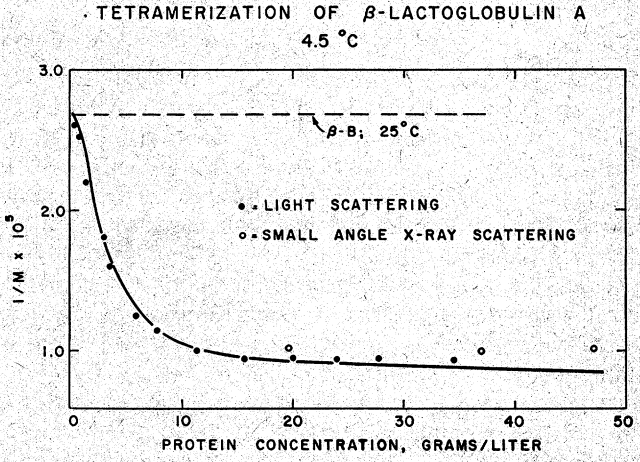

Figure 6. Scattering data on β-lactoglobulin A at 4.5°C, pH 4.65, 0.1 ionic strength acetate buffer (conditions of maximal tetramerization). Filled circles: light scattering (ref. 50); open circles: small angle X-ray scattering (ref. 36). For comparison the light scattering data on β-lactoglobulin B at 25°C (no aggregation) are shown by the dashed line.

The dependence of the activity coefficient on the state of aggregation of a macromolecule has permitted studies on specific aggregation reactions and heterogeneous associations between different chemical species. Light scattering has been used to characterize the thermodynamics of a number of polymerizing protein systems, suffice it to mention insulin (*45*, *46*), mercaptalbumin (*47*) and β-lactoglobulin (*48*, *49*), while the highly important antigen-antibody reaction was studied by Pepe and Singer (*50*). A plot of typical light scattering and small angle X-ray scattering data obtained with β-lactoglobulin under conditions at which it exists in a monomer-tetramer equilibrium is shown in Figure 6. In this plot, the experimental points are values of the weight average molecular weight of the system as

a function of concentration. From such data, association equilibrium constants can be obtained immediately, leading to the thermodynamic parameters. Of particular importance in this figure is the presence of both kinds of scattering data. The two sets of points are seen to be in good agreement demonstrating the practicability of using the two techniques simultaneously in thermodynamic studies. This is the first instance of the simultaneous application of the two techniques to a thermodynamic problem.

When the macromolecules are dissolved in the presence of supporting electrolyte, it becomes necessary to use the three-component theory (equation (9)) in the data analysis. For this, independent knowledge of protein-small ion and small ion-small ion interactions is required. In the last two years, the Stockmayer-Kirkwood-Goldberg multicomponent theory has been extended (*51*), showing that the extra interaction measurements can be avoided in light scattering if the solution is first dialyzed against the solute containing the third component and the refractive increment and excess scattering intensity are measured against the dialysate. In such cases, equation (9) reduces to equation (7). While simplifying the situation when component 1 is dialyzable, this approach does not preclude the rigorous use of equation (9) when this component is also nondialyzable (*52*), for example in the case of protein-protein interactions.

A further very important source of information is the slope (second virial coefficient) of the scattering function as a function of concentration. If supporting electrolyte is present the problem has been treated in 1959 by Stigter and Hill (*53*) who used the cluster theory of McMillan and Mayer (*54*). Since frequently it is necessary to work without extrapolation to zero concentration, knowledge of the contribution of virial effects to scattering becomes necessary. For a globular polyelectrolyte, such as a protein, the electrostatic contribution to the coefficient of C_2 in equation (7) or (9) can be calculated using the method of Stigter and Hill (*53*) or the essentially equivalent form (*55*):

$$\frac{1}{RT}\left(\frac{\partial \mu_2^e}{\partial C_2}\right) = -\frac{4\pi N}{M_2}\int_0^\infty \left\{\left[\exp -\left(\frac{\overline{Z}_2 e^2}{DRkT}\,\frac{e^{-\kappa(R-a)}}{1+\kappa a}\right)\right] - 1\right\} \cdot R^2 dR + \frac{7\pi N b^3}{6M_2} \quad (12)$$

where N is Avogadro's number, e the electronic charge, R the distance from the center of a given molecule, D the dielectric constant, b the radius of the protein molecule considered as a sphere, κ the reciprocal of the Debye-Hückel double layer thickness, and a the center-to-center distance of closest approach between the protein molecule and the small ion. The second term of this equation represents the excluded volume. The development of this theory has generated a great deal of activity leading to the examination of systems, which can serve as models for proteins.

Before passing on to problems of molecular geometry, it should be pointed out that protein molecular weights have been determined recently by small angle X-ray scattering (*56*, *57*) and, with the present availability of absolute intensity techniques, a burst of X-ray scattering activity might be expected in the field of biopolymer thermodynamics.

While the first applications of light scattering to biological macromolecules were in the realm of molecular weight measurements and thermodynamic studies, its use for the measurement of the size and shape has also developed rapidly, especially after the introduction in 1948 by Zimm (*18*) of his double extrapolation plot. In the Zimm approach, equations (4) and (7) are combined to give

$$K'\frac{C_2}{\Delta i_s} = \frac{1}{M_2}\left[1 + \frac{1}{RT}\frac{\partial \mu_2^e}{\partial C_2}C_2 + \frac{16\pi^2}{3\lambda^2}(R_G)^2 \sin^2\theta/2 + \ldots\right] \quad (13)$$

In this method, extrapolation to zero concentration results in a line, the limiting slope of which as function of $\sin^2\theta/2$ gives the radius of gyration.

In small angle X-ray scattering it has been customary to use directly the Guinier equation (*7*) (equation (4)), for obtaining R_G. A typical example of such a plot is shown in Figure 5. A number of applications of this latter technique can be found in the literature, the earliest work appearing in the studies of Guinier and Fournet (*4*), Kratky and Porod (*58*), and Beeman and co-workers (*59*).

In the past, due to instrumentation problems, small angle X-ray scattering has been limited to a few groups of workers, while the light scattering technique for measuring molecular geometry has been very widely applied. Light scattering, using the Zimm approach, has been very important in the characterization of such biopolymers as DNA (*6*), muscle proteins (*6*), collagen (*60*), TMV (*61*), synthetic polypeptides (*62*), and starch (*63*). For different molecular structures (spheres, rods, coils, etc.), the radius of gyration is a different function of the molecular weight. This difference can be used to advantage in the characterization of the detailed shape of macromolecules. Since an excellent review of this problem exists in the recent article by Geiduschek and Holtzer (*6*), it will not be taken up here. In applying the Zimm equation (13), it must be emphasized that its validity is limited to the region in which $(R_G)^2 \sin^2(\theta/2)$ is small. Thus, for very large molecules, meaningful values of R_G and M_2 can be obtained only from measurements at very low angles. As pointed out by Sadron (*64*) in 1958, this fact had introduced a great deal of doubt about the validity of measurements on DNA, but the recent development by Katz (*65*) and by Froelich (*66*) of equipment capable of measurements down to 5° should solve this particular problem.

As a typical example of the structural problems attacked by scattering techniques, one should cite that of the structure of synthetic polypeptides in solution. This is of extreme importance since it bears on the question of the existence of the α-helix in solution, as well as in fibers. As a result of a light scattering study, Doty, Bradbury, and Holtzer (*62*) reported in 1956 that in certain solvents poly-γ-benzyl-L-glutamate exists in the form of a rigid rod whose dimensions agree quantitatively with an α-helix. Luzzati and co-workers (*67*) reexamined this problem in 1961 using the method of small angle X-ray scattering, which permitted them to measure the mass per unit length and radial radius of gyration of the polypeptide rods. While they agreed that indeed this material exists in the form of rods in solution, they concluded that the rod is not an α-helix but a 3-10 helix. Since similar syn-

hottic polypeptides have been used as standards for teher techniques for measuring the amount of α-helix in a protein molecule in solution (such as optical rotation dispersion (*68*)), the effect of this disagreement is to cast a serious doubt on the validity of much research done in the last few years. As a result, a vast amount of activity is being devoted now to the resolution of this question. In a recent publication, Benoit and co-workers (*76*) have described the results of a careful hydrodynamic examinations of this problem with results in good agreement with the small angle X-ray scattering conclusions (*67*). Both types of helices have been found in the birefringence and optical rotation dispersion experiments of Tinoco and Yamaoka (*77*).

Besides reopening the question of the solution structure of polypeptides, recent developments in small angle X-ray scattering techniques have led to the examination of the structures of a number of other biopolymers. Thus Kratky has concluded that cellulose nitrate (*69*) and rubber (*70*) fit best the geometry of worm-like chains. Luzzati and co-workers (*56*) have carried out a study of the acid expansion of bovine serum albumin, as a result of which they have challenged the validity of structural models proposed for this protein from a vast amount of hydrodynamic and optical data (*71–73*). Furthermore, while small angle X-ray scattering has confirmed the existence of the Watson-Crick double helix in DNA in solution (*38*), it has also established its presence in microsomal RNA (*74*). In the last case, light scattering and small angle X-ray scattering measurements on the same material have been found to fall on the same geometric plot, confirming their complementarity (*52*); a combination of the internal structure seen by X-ray scattering with the overall structure given by light scattering has led to a model capable also of accounting for the hydrodynamics (*75*) of this genetically important macromolecule.

Conclusion

From the preceding remarks, it can be seen that each development in the theory or instrumentation of light and small angle X-ray scattering has been followed by a burst of activity in the field of biological macromolecules. This has resulted in an ever-increasing understanding of the mechanisms of association of biopolymers and of their detailed structures in solution.

In these brief considerations of the development of the two scattering techniques, it has been shown by examples how these methods have influenced and are still influencing the development of our understanding of large biological molecules. Conversely, new discoveries in biology have also greatly influenced the development of these two techniques, asking them for answers to ever more complicated questions. While no attempt has been made to review their entire history, it is hoped that an inkling can be gained of the vast contribution which they have made, particularly in the last 15 years, to our understanding of macromolecules of biological origin.

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